Synopsis of Thesis in Title

Phytochemical Analysis and potential Theraputic Screening of *Garcinia cambogia L.* and *Averrhoa carambola* L.

To be submitted for registration of

Ph.D. in Botany

Under the Faculty of Science and Technology

To

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By

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1) Introduction:

Phytochemicals are bioactive nutrient of plant chemicals in fruits, vegetables, grains and other plant food that may provide desirable health benefit beyond basic nutrient to reduce the risk of chronic disease (Liu, 2004).They includes phenols, phenolic acids, flavonoids, terpenoides, carotenoids, etc. They are naturally occurring, responsible for providing colour, flavor, aroma to fruits and vegetables along with help plant protect against disease, infection, etc. also help in curing an animal disease.

Plant-derived medications are regarded as the first line of defense in sustaining health and battling diseases, and plant sources continue to be the primary source of new therapeutic drugs used today (Samy*et al*., 2008). This study aims to characterize the phytochemicals, therapeutic activity and antioxidant activity of different solvent extract from selected medicinal plants.

An antioxidant is a molecule that inhibits the oxidation of other molecules. Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness. (Yadav , Kumari, & Yadav , 2016). Phenolic, flavonoids, lycopene, carotenoids and glucosinolates are among the most thoroughly studied antioxidants (Charanjit Kaur and Harish C. Kapoor2010).The DPPH antioxidant profiling is use for evaluating antioxidant activity.

Cancer has become more prevalent in recent times throughout the World next to diabetes. The uncontrolled cell division leading to extra mass in tissue/tissues is called cancer. However, all the extra masses formed are not cancerous growths. These extra masses are called tumours.Tumours are two types, 1.Benign Tumours (Benign tumours are not cancers, they can be removed). 2. Malignant Tumours (Malignant tumours are cancers, here cells divide abnormally without any control).

According to cancer research U.K, there are about 60 different organ tissues in the body where cancer can originate. The most common two type of cancers in India are Breast Cancer & Cervical Cancer. More than 1 hundred different types of cancer have been recognized and twenty four different types of cancer occur in different parts of human bodies. (Wani & Nair, 2003). Doctor divide cancer into types based on where it beings. Four main types of cancer are: Carcinoma: Related to skin or tissue that covers the surface of internal organ and glands, (e.g., prostate cancer, breast cancer, lung cancer, colorectal cancer). Sarcomas: A sarcoma begins in the tissues that support and connect the body. (e.g., bone cancer) Leukemias: Leukemia beings when healthy blood cells change and grow uncontrollably (e.g. Blood cancer).

Lymphomas: Lymphoma is a cancer that beings in lymphatic system. (E.g. CLL- chronic lymphocytic leukemia)

Urban Indian population is now the most commonly diagnosed with cancer. The reason for the recent observed increase in incidence of cancer in Indian population are not clearly understood but thought to be largely explained by 'Westernization' of lifestyles and changes in reproductive behaviour, compared to developed countries overall there were less cancer cases in India but that could be due to under diagnosis and under reporting. At the same time, regional, ethnic, dietary and socio-economic factors might also result in difference in the cancer susceptibilities and the incidence. In addition, cancer was mainly a disease of old ages. Worldwide median age at diagnosis was about 60 years. Average life span was about 58 yrs. in India compared to 75 yrs. in the developed world.

In recent years, oxidative stress and the mechanisms by which cancer is cause has been extensively studied. Cancer development is a multistage process which involves mutations in critical genes required for maintenance of the cellular homeostasis. Oxidative stress causes initiation, promotion and progression of carcinogenesis. ROS play an important role in the development of cancer. Oxidative damage to DNA or of antioxidant defence systems leads to mutation, activated transcription factors, modification of gene expression and chromosomal aberrations, processes that have been describe as the agents of progressions of cancer. There are many evidences that the generation of free radicals inside the body cause damages to DNA and lead to the development of cancer, etc., and if these free radicals are neutralized by the antioxidants from different medicinal plants, then it prevents cancer. Several studies have shown that plant derived antioxidant scavenge free radicals and modulate oxidative stress.

2) Objectives:

1. Collection, identification and authentication of selected medicinal plant.
2. Preparation of plant extracts by Soxhlet method.
3. Phytochemical screening of selected plant extracts.
4. Evaluate the anti-oxidant potential of selected medicinal plants.
5. Evaluate the potential theraputic activities.

3) Review of literature:

1. (Ghosh & Sinha , 2014). They determined the total phenolic content by using the Folin-Ciocalteu reagent and aluminium chloride colorometric method used for the determination of total flavonoids, in *litsea polyantha* Juss bark, and the result obtained showed that the plant material studied is a good source of phenolic and flavonoid compounds.

2. (Catherine , Nicholas, & Bolwell, 1995). There article discusses the biological properties of the flavonoids and focusses on the relationship between their antioxidant activity, as hydrogen donating free radical scavengers, and their chemical structures. This culminates in a proposed hierarchy of antioxidant activity in aqueous phase. The cumulative findings concerning structures- antioxidants in the lipophilic phase derive from studies of fatty acids, liposomes, and low density lipo proteins: the factors underlying the influence of the different classes of poly phenols in enhancing their resistance to oxidation are discussed and support the contention that the partition coefficients of flavonoids sa well as their rates of reaction with the relevant radicals define the antioxidant activity in the lipophilic phase.

3. (Li Fu, Bo-Tao Xu, & Yuan Zhang, 2011). Studied the antioxidant activity and total phenolic contents of 62 fruits and were evaluated using ferric reducing antioxidant power (FRAP). Trolox equivalent antioxidant capacity (TEAC) assays as well as Folin-Ciocalteu method respectively. The correlation between FRAP value and TEAC value as well as total phenolic content were also assessed. The result showed that different fruits had diverse antioxidant capacities.

4. (Yadav, Kumar, & Vihan, 2010). They studied indigenous plant to explore the phytochemical constituents. About 7 indigenous plants collected from Agra- Mathura region. The collected extraction followed by evaporation. The prepared plant extract goes through phytochemical investigation to explore active constituents which are very significant in drug development.

5. (Hua-Bin Li & Chi-Chun Wong, 2008). Studied the antioxidant capacity of 45 medicinal plants and were evaluated using ferric reducing antioxidant power (FRAP) and Rolex equivalent antioxidant capacity (TEAC) assays respectively and total phenolic content of this plants were measured by the Folin-Ciocalteu method. Most of these plants were analyzed for the first time their antioxidant activities.

6. (Islam & Nasrin , 2013). Their research is directed towards finding naturally occurring antioxidants having (Rod).

7. (Srivastava, Tiwari, & Sharma, 2013). Studied the assessment of phenol and flavonoid content in the plant materials.

8. (Kale M S, Laddha K S, (2012). They determined the total flavonoid content and quantitative estimation of rutin in methanolic extract of *Memordica tubersa(Roxb)* cogn. Fruits were

performed. The spectrophotometric method based on the formation of complexes with aluminium chloride was applied to determine the total flavonoid content. Presence of rutin was identified using chemical test and thin layer chromatography (TLC).

9. **(Ramamoorthy & Bono, 2007)**. Studied the antioxidant activity, total phenolic and flavonoid content of *Morinda citrifolia* fruit extracts from various extraction process.

10 (Sumaira Sahreen, Khan, & Khan, 2010). Studied the evaluation of antioxidant activities of various solvent extracts of *Carrica apaca* and verified that the chloroform and aqueous fraction have strong antioxidant activities which were correlated with its high level of phenolics and flavanoids. These fractions can be used as a source of potential antioxidant or functional food material.

11. (Villano , Fernandez-Pachon, & Troncoso, 2005). Studied the comparison of antioxidant activity of wine phenolic compounds and metabolites in vitro.

12. (John & Najla, 2002). Citrus fruits contain high concentration of several classes of phenols, including numerous hydroxycinnamates, flavonoids glycosides, and polymethoxylated flavones. The latter group of compound occurs without glycoside linkage and has been shown to inhibit the proliferation of a number of cancer cell lines. This antiproliferative property was further demonstrated against additional human cancer cell line and the antiproliferative action of series of synthetic methoxylated flavones were also studied. Similar to the naturally occurring compounds, the synthetic compounds exhibit strong antiproliferative activities. In many causes the IC_{50} values occurred below 10um. Other hydroxylated flavones and flavanone aglycons also exhibit antiproliferative activities against cancer cell line, with the flavones showing greater activities than flavanones. Glycosylation of these compound removed their activity. The strong antiproliferative activities of the plymethoxylated flavones suggest that may have use as anticancer agents in humans.

13. (Wenying , Qiao, Wang, & Zhu, 2003). The role of dietary flavonoids in cancer prevention is widely discussed. Compelling data from laboratory studies, epidemiological investigation, and human clinical trials indicates that flavonoids have important effect on cancer chemopreventation and chemotherapy. Many mechanism of action have been identified, including carcinogen inactivation, anti-proliferation, cell cycle arrest, induction of apoptosis and differentiation, inhibition of angiogenesis, antioxidant and reversal of multidrug resistance or a combination of these mechanism. Based on these result, flavonoids may be promising anticancer agent

4) Materials and Methods:

4.1) Collection of medicinal plant from local area.

4.2) Preparation of plant extracts by soxhlet method using suitable solvent: The plant material collected was washed under tap water and cut into slices. The dried slices were grinded and made into powder, 20 gm of plant powder was extracted using soxhlet apparatus. The solvents used for extraction were ethanol and Methanol. The temperature was maintained in each extraction was 20˚C lower the melting point of the solvents. The extract was filtered, concentrated by boiling, dried and is stored in refrigerator for further use.

4.3) Qualitative and quantitative phytochemical analysis of selected medicinal plant.

4.3.1) Qualitative Phytochemical analysis:

Qualitative screening of all the extracts of all the solvents i.e., ethanol and distil water were carried out to detect the presence of various phytoconstituents like proteins, carbohydrate, phenols, flavonoids, glycsosidases, steroids, fixed oils, phlobatanins etc. (Yadav & Agarwala, 2011).

Test for proteins, (Ninhydrin test):

Crude extracts when boiled with 2 ml of 0.2 % solution of Ninhydrin, violet colour appeared suggesting the presence of amino acids and proteins.

Test for carbohydrates, (Benedict's test):

Extract when mixed with 2 ml of Benedict's reagent and boiled, a reddish-brown precipitate formed which indicated the presence of the carbohydrates.

Test for phenols and tannins (Ferric test):

Extracts were mixed with 2 ml of 2 % solution of FeCl3 respectively. A blue-green or black coloration indicated the presence of phenols and tannins.

Test for flavonoids (Alkaline reagent test):

Each extracts were mixed with 2 ml of 2 % solution of NaOH respectively. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

Test for glycosides (Keller-Kilani test):

Extracts were mixed with 2 ml of glacial acetic acid containing 1-2 drops of 2 % solution of $FeCl_3$. The mixture was then poured into another test tube containing 2 ml of concentrated

H_2SO_4. A brown ring at the interphase indicated the presence of cardiac glycosides.

Test for steroids:
Extracts were mixed with each of 2 ml of chloroform and concentrated H_2SO_4 and was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids.

Test for fixed oil and fatty acids (Spot test):
Prepared spot on the filter paper with the test solution and oil staining on the filter paper indicated the presence of fixed oil and fats.

Test for Phlobatannins:
About 2 ml of aqueous extracts were added to 2 ml of 1 % HCl and the mixture was boiled. Deposition of a red precipitate was taken as evidence for the presence of Phlobatannins.

4.3.2) Quantitative Phytochemical Analysis:

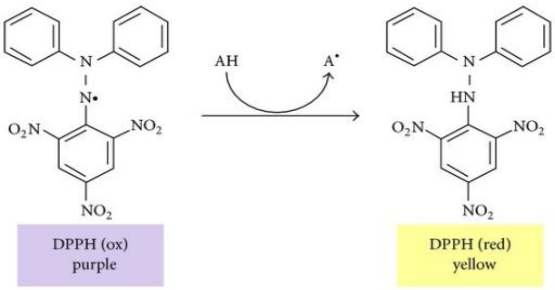
Quantitative estimation Phenols: (Sudakshina Das et al, 2015)

Total Phenolic content was analyzed by using standard method with little modifications. 10mg of the both extracts (ethanol and aqueous) of the sample was weighed accurately and dissolved in 10 ml of respective solvents. 1 ml of this solution was transferred to a test tube, then 0.5 ml of Folin-Ciocalteu reagent was added and vortexed. After 5 minutes, 2 ml 20% of Na2CO3 solution was added and ultimately the volume was made up to 8 ml with distilled water and finally incubated for 30 minutes at room temperature. After Incubation, the absorbance against the reagent blank was determined at 765 nm. These data were used to estimate the total phenolic content using a standard calibration curve obtained from various diluted concentrations of Gallic acid. The total phenolic content of the plant was expressed as Gallic acid (equivalent mg/g of extracted compounds). Samples were analyzed in triplicates.

Quantitative estimation of Flavonoids: (Kalita, Tapan, & Pal, 2013)

Aluminium chloride colorimetric method was used with some modification to determine flavonoid content. To the each extract solution, 1.5 ml methanol, 0.1 ml aluminium chloride, 0.1 ml potassium acetate solution and 2.8 ml distilled water were added and mixed well and incubated for 30 min. at room temperature. The absorbance was measured at 415 nm. Quercetin was used as standard (1mg/ml). Flavonoid content were calculated from standard curve obtained from various concentrations of Quercetin and was expressed as quercetin equivalent (mg/g).

4.4) Evaluation of antioxidant potential of selected plant.



DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH. The color changes from purple to yellow after reduction, which can be quantified by its decrease of absorbance at wavelength 517 nm. Radical scavenging activity increased with increasing percentage of the free radical inhibition. The degree of discoloration indicates the free radical scavenging potentials of the sample/antioxidant by their hydrogen donating ability.

Determination of DPPH radicals scavenging activity:

The free radical scavenging activity of the extract was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH. Determination of DPPH radicals scavenging activity was estimated with the method used by (Ansari , Ahmed, Waheed, & Juned, 2013) with slight modification.

DPPH radical scavenging activity (%) =

(Absorbance of Control – Absorbance of test sample)/ Absorbance of control × 100

4.5) Evaluation of potential theraputic activities:

In potential therapeutic screening main focus on anticancer activites, antimicrobial and antihyperglasemic, etc.

For evaluation of this activities standard protocol will be follow.

5) Importance of study/ Society application

International status: As per GLOBOCAN 2020 worldwide, an estimated 19.3 million new cancer cases and almost 10.0 million cancer deaths. Lung cancer remained the leading cause of cancer death, with an estimated 1.8 million deaths (18%), followed by colorectal (9.4%), liver (8.3%), stomach (7.7%) and female breast (6.9%). Overall incidence was from 2-fold to 3-fold higher in

transitioned versus transitioning countries for both sexes where mortality varied. A global cancer burden is expected to be 28.4 million cases in 2040, a 47% rise from 2020. (Hyuna Sung, et.al. 2021).

National status: Cancer cases in India increased at an average annual rate of 1.1-2% from 2010-2019, according to new report. Deaths from cancer in the country also went up. As many as 1,392,179 people in India had cancer in 2020, according to PBCRs (Population based cancer registries).Five leading sites of the cancer is breast, lung, mouth, cervix, uteri and tongue. New cancer cases jumped to over 23million in 2019 from 18.7million in 2010. Cancer death reached 10million and 26.3%.in 2019 from 8.29million in 2010.this figure represent an increase of 20.9% and 26.3%.

Significance of study:

The result of the proposed research work may useful for the preparation of effective herbal medicine. The output of the study will enhance the knowledgebase of Indian Phytochemicals as potential therapeutic agents which can be used to treat and manage several dreadful diseases like cancer.

6) Proposed work plan /formulation and structure of study.

Year wise plan of work and targets to be achieve.

Year I: Literature search and standardization of experimental protocols

Year II: Collection and study of selected different plant samples on Cancer cell line, anti-oxidant studies, cytotoxicity studies

Year III: Thesis writing and publishing in journals of repute.

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